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Case–control study of genus-beta human papillomaviruses in plucked eyebrow hairs and cutaneous squamous cell carcinoma

Michelle R. Iannacone[1], **Tarik Gheit**[1], **Herbert Pfister**[2], **Anna R. Giuliano**[3], **Jane L. Messina**[4], **Neil A. Fenske**[5,6], **Basil S. Cherpelis**[5,6], **Vernon K. Sondak**[4], **Richard G. Roetzheim**[7], **Steffi Silling**[2], **Michael Pawlita**[8], **Massimo Tommasino**[1], and **Dana E. Rollison**[9]

[1]Infections and Cancer Biology (ICB) Group, International Agency for Research on Cancer, Lyon Cedex, 08 France

[2]Institute of Virology, University of Cologne, Cologne, Germany

[3]Center for Infection Research in Cancer, Moffitt Cancer Center, Tampa, FL

[4]Cutaneous Oncology Program, Moffitt Cancer Center, Tampa, FL

[5]Department of Pathology and Cell Biology, University of South Florida College of Medicine, Tampa, FL

[6]Department of Dermatology and Cutaneous Surgery, University of South Florida College of Medicine, Tampa, FL

[7]Department of Family Medicine, University of South Florida College of Medicine, Tampa, FL

[8]Infection and Cancer Program, German Cancer Research Center Deutsches Krebsforschungszentrum, DKFZ Heidelberg, Germany

[9]Department of Cancer Epidemiology, Moffitt Cancer Center, Tampa, FL

Abstract

Cutaneous human papillomaviruses (HPV) have been reported in cutaneous squamous cell carcinoma (SCC). We conducted a clinic-based case–control study to investigate the association between genus-beta HPV DNA in eyebrow hairs (EBH) and SCC. EBH from 168 SCC cases and 290 controls were genotyped for genus-beta HPV DNA. SCC tumors from a subset of cases (n = 142) were also genotyped. Viral load was determined in a subset of specimens positive for a single HPV type. Associations with SCC were estimated by odds ratios (OR) and 95% confidence intervals (CI) adjusted for age and sex using logistic regression. Statistical tests were two-sided. EBH DNA prevalence was greater in cases (87%) than controls (73%) ($p < 0.05$), and the association with SCC increased with the number of HPV types present, (≥4 types *vs*. HPV-negative: OR = 2.02, 95% CI = 1.07–3.80; $p_{trend} = 0.02$). Type-specific associations were observed between SCC and DNA in EBH for HPV23 (OR = 1.90, 95% CI = 1.10–3.30) and HPV38 (OR = 1.84, 95% CI = 1.04–3.24). Additionally, when compared with the controls, the DNA prevalence in EBH was significantly higher among cases for 11 of the 25 genus-beta types

Correspondence to: Dana E. Rollison, Department of Cancer Epidemiology, Moffitt Cancer Center, 12902 Magnolia Drive, Tampa, FL 33612, USA, Tel.: +813-745-6530, Fax: +813-745-6525, dana.rollison@moffitt.org.

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tested, when accounting for DNA for the same HPV type in the tumor (ORs = 3.44–76.50). Compared to controls, the mean viral DNA load in EBH among the selected cases was greater for HPV5, HPV8 and HPV24, but lower for HPV38. SCC cases were more likely than controls to have HPV DNA+ EBH for single and multiple HPV types, providing additional support for the potential role of genus-beta HPV infections in SCC development.

Keywords

case–control; squamous cell carcinoma; genus-beta human papillomaviruses; eyebrow hairs; DNA

Epidemiological evidence supports a potential role for cutaneous human papillomaviruses (HPV) in the development of squamous cell carcinoma (SCC). The presence of genus-beta HPV DNA in SCC tumor tissues has been reported among immunocompetent individuals, with prevalence ranging from 20 to 48%.[1-6] Genus-beta DNA has also been detected in plucked eyebrow hairs from SCC cases with prevalence estimates varying by country (44–90% in Australia,[7-9] 71–93% in The Netherlands[6,7,10] and 95% in Italy[7]). In addition, SCC has been positively associated with DNA for any genus-beta type in eyebrow hairs[6-8,10] as well as specific types 5, 15 and 20.[6,10]

Highly sensitive PCR techniques[11] enable quantification of viral DNA in human tissue samples and may serve as an indicator for production of virions. In contrast to the high viral DNA loads observed in patients with epidermodysplasia verruciformis (EV),[12] and in organ transplant recipients,[13] lower viral DNA loads in eyebrow hairs have been observed among immunocompetent individuals without a history of SCC[13] suggesting the potential importance of the immune system in prevention of HPV infection and SCC development. However, epidemiologic studies investigating the association between HPV activity and SCC among immunocompetent individuals have been limited.

We previously reported that genus-beta HPV seropositivity was associated with SCC and that, compared to controls, genus-beta HPV seroprevalence was greater among SCC cases with genus-beta HPV DNA positive tumors.[14] This analysis investigated the association between genus-beta HPV DNA in plucked eyebrow hairs and SCC within the same case–control study population. To our knowledge, this is the first epidemiological study in a US population to investigate the association between genus-beta HPV DNA in eyebrow hairs and SCC and to stratify such estimates by the presence or absence of genus-beta HPV DNA in the tumor tissues among the SCC cases. We also measured genus-beta typespecific HPV DNA load in eyebrow hairs and SCC tumor tissues for a subset of participants.

Material and Methods

Study design and population

The clinic-based case–control study design and population have been previously described in detail.[14,15] Briefly, histologically confirmed SCC cases (n = 191) were recruited from the University of South Florida (USF) Dermatology clinic. Control subjects comprised patients undergoing skin cancer screening exams at Moffitt's lifetime cancer screening (LCS) and patients undergoing routine physical examinations at the USF Family Medicine clinics.

Controls had no history of any cancer and were determined to be free of prevalent skin cancer based on full-body skin cancer screening exams (n = 281). If a patient's suspicious lesion detected during the exam was determined to be benign based on pathology review, the patient was included as a control (n = 77). If a patient's screen-detected lesion was histologically confirmed to be an SCC, then that patient was included as a case (n = 6). All study participants were aged 18–80.

Demographic and sun exposure-related characteristics for study participants were captured by questionnaire. With the exception of two non-White controls, all participants were White. At the time of study enrollment, six to eight eyebrow hairs were plucked from study participants and snap frozen in liquid nitrogen. Of the 174 SCC cases and 300 controls with available cutaneous HPV serology data,[14] eyebrow hair samples were available from 169 cases and 295 controls. After exclusion of beta-globin negative specimens, the final sample size for the analysis of HPV DNA in eyebrow hairs was 168 cases and 290 controls.

A 3-mm, flash frozen punch of tumor tissue was obtained from SCC patients. Only beta-globin-positive specimens were included, corresponding to 180 tumors from 159 individuals, including 19 who contributed tissues from distinct, concurrent tumors. The final sample size for analyses including HPV DNA in eyebrow hairs and DNA status of the tumors consisted of 142 cases and 290 controls. Written informed consent was provided by all study participants after all study procedures were approved by the institutional review board at USF.

DNA extraction and HPV genotyping

DNA extraction from fresh-frozen SCC tumor tissues and plucked eyebrow hair samples was conducted with the QIAGEN EZ1 DNA Tissue Kit. HPV genotyping was performed, blinded to case–control status, by a type-specific multiplex genotyping (TS-MPG) assay.[16-19] Multiplex-PCR was performed using serial dilutions of HPV DNA (from 1,000 to 0 copies of viral genome) from different beta HPV types as the template. PCR products were obtained even when only ten copies of the viral genome for each HPV type were used as a template. HPV genotyping was successfully repeated in a blind manner, three times in ten individual subjects, demonstrating reproducibility for specific HPV types.[16] The assay detects the DNA of 25 genus-beta HPV types (5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 49, 75, 76, 80, 92, 93 and 96). Two primers for the amplification of beta-globin were added to provide a positive control for the quality of the template DNA.[20] Information on DNA positivity for HPV49 was not available for the tumor tissues.

Quantitative, real-time PCR

Detection of the beta-globin gene and the number of DNA copies of HPV5, 8, 15, 20, 23, 24, 36 and 38 in selected eyebrow hair and tumor tissue samples was conducted, blinded to case–control status, by quantitative PCR (qPCR) using the "LightCycler-Control Kit DNA" (Roche) and protocols described previously.[11,21] Replicate assays of samples with viral loads of 2–100 HPV DNA copies per 2 μl showed high reproducibility with a maximal deviation of 66% and an average of ±21%.

SCC cases infected with an identical, single genus-beta HPV type in both their eyebrow hair and tumor samples (as previously determined by multiplex-PCR) were selected for viral DNA load determination ($n = 31$). For comparison, controls were also selected for viral load analysis ($n = 56$). Controls were chosen if they had a single genus-beta HPV infection in their eyebrow hairs that was the same single HPV type detected in some SCC cases. For example, HPV5 was detected by multiplex PCR as a single infection in both the eyebrow hair and tumor for four SCC cases. HPV5 viral load was subsequently measured in eyebrow hairs and tumors for these four SCC cases, in addition to the eyebrow hairs of five controls that also tested positive only for HPV5 by multiplex PCR.

Statistical analysis

The chi-square test was used to compare skin cancer risk factors between cases and controls. Genus-beta HPV typespecific DNA prevalence was calculated as the proportion of SCC cases and controls who tested positive for DNA to a given type. Overall HPV DNA prevalence was calculated as the proportion of patients who tested DNA-positive for at least one HPV type. Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the associations between HPV DNA positivity in eyebrow hairs and SCC. To account for multiple comparisons, the Bonferroni correction method was applied, reducing the significance level to $p < 0.002$. Associations between sunlight related factors and SCC were stratified by the presence or absence of HPV DNA in the eyebrow hairs, and stratum-specific ORs and 95% CI were estimated. These factors included history of blistering sunburn (yes *vs*. no), cutaneous sensitivity to the season's first sunlight exposure (sunburn with or without blistering *vs*. mild sunburn that turns to a tan/tan/no change in skin color) and tanning ability to repeated sunlight exposure (tans easily *vs*. unable to tan/tan after working at it). Statistical significance of multiplicative interactions between genus-specific HPV DNA status in the eyebrow hairs and sunlight-related factors as they related to SCC was tested by placing an interaction term for the product of HPV DNA status and each sunlight related factor in the logistic regression models. A p-value of < 0.05 for the beta coefficient corresponding to the interaction term was considered statistically significant.

SCC tumor tissues were classified as positive or negative for the presence of genus-beta HPV DNA. The 19 cases who contributed more than one SCC tumor tissue were considered DNA-positive if at least one of the tumor tissues provided tested positive for genus-beta HPV DNA. Type-specific concordance was calculated among the SCC cases as the proportion that tested DNA-positive for a given HPV type in the eyebrow hairs and who also had DNA in their tumor tissue corresponding to the same HPV type. Logistic regression was used to estimate the OR and 95% CI corresponding to the case–control differences in type-specific DNA positivity in the eyebrow hairs, stratified by the presence or absence of DNA to the same HPV type in the SCC tumor tissue.

Factors that altered the study-specific ORs and 95% CIs by more than 10% were adjusted for in the logistic regression models (*i.e*., age, sex, education, hair color, occupational sunlight exposure, tanning ability and smoking). To rule out the possibility of residual confounding by sex and age, independent analyses were conducted stratified by sex and restricted to a narrower age range of 40–69 years.

Type-specific HPV DNA load for eyebrow hair and tumor tissue samples was quantified by dividing the number of copies of HPV DNA detected in each sample by the total number of cells (determined by the number of beta-globin copies divided by two) in that sample. In view of low viral DNA loads (<1 viral DNA copy per cell), mean viral DNA loads were expressed as the number of viral copies per 100,000 cell equivalents. Mean viral DNA load comparisons excluded samples that did not amplify the beta-globin gene. Within SCC cases, viral DNA load comparisons between eyebrow hairs and tumors were restricted to include only those cases that were determined to be qPCR positive in both samples. Case–control comparisons of viral DNA loads in eyebrow hairs were restricted to HPV types where at least one case and one control were positive for that type (5, 8, 23, 24 and 38).

To examine the associations between HPV eyebrow hair DNA and SCC in the context of our previously published HPV serology results,[14] we conducted two stratified analyses. The first was modeled after the study by Proby *et al.*[22] to facilitate comparisons across studies. The second was designed to tease apart the independent and combined effects of HPV seropositivity and HPV DNA in eyebrow hairs by defining those who were HPV seronegative and DNA negative as the reference group.

All statistical tests were considered two-sided. Analyses were performed using the SAS statistical software package (version 9.2; SAS Institute).

Results

Demographic characteristics between cases and controls are presented in Table 1. SCC cases tended to be older ($p < 0.0001$), male ($p < 0.0001$), less educated ($p = 0.001$) and smokers ($p = 0.0002$). Cases also exhibited lighter phenotypic characteristics, such as light eye ($p = 0.003$) and hair ($p = 0.003$) color, displayed greater cutaneous sensitivity ($p = 0.001$) and an inability to tan ($p < 0.0001$) during sunlight exposure, and were more likely to report a history of occupational sunlight exposure ($p < 0.0001$).

Compared to 73% of controls, 87% of SCC cases had genus-beta DNA positive-eyebrow hairs. After adjustment for age, sex, education, hair color, occupational sunlight exposure, tanning ability, and ever-smoking status, type-specific analyses revealed significant associations between SCC and DNA in eyebrow hairs for HPV23 (OR = 1.90, 95% CI = 1.10–3.30) and HPV38 (OR = 1.84, 95% CI = 1.04–3.24) (Table 2). SCC was associated with an increase in the number of genus-beta types present in the eyebrow hairs, with positivity up to ≥4 types associated with a twofold risk of SCC (OR = 2.22, 95% CI = 1.07–4.61; $p_{trend} = 0.03$) (Table 2). Stratified analyses demonstrated no significant differences by sex in the associations between HPV DNA in eyebrows and SCC (data not shown). When analyses were restricted to individuals aged 40–69, similar associations were observed for HPV23 and HPV38 (Table 2). No associations presented in Table 2 remained statistically significant after accounting for multiple comparisons.

Associations between measures of sunlight exposure and SCC did not differ significantly by the presence or absence of HPV DNA in eyebrow hairs, including history of blistering sunburn ($p_{interaction} = 0.10$), cutaneous sensitivity to the season's first sunlight exposure

($p_{interaction}$ = 0.30), and tanning ability to repeated sunlight exposure ($p_{interacton}$ = 0.40) (data not shown).

Sixty-six percent of SCC tumors were DNA-positive for ≥1 genus-beta HPV type(s) (Table 3). Tumors developed mostly in the head and neck region (56.1%) compared to other body parts (43.9%) (data not shown). With the exception of HPV15 (p = 0.001), the prevalence of individual HPV types did not vary by anatomical region of tumor development (data not shown). Among the 19 SCC cases that contributed more than one tumor specimen, HPV type-specific DNA positivity across tumor tissues and eyebrow hair samples were compared. Eleven of nineteen cases tested positive for at least one identical type across tumor samples and ten of nineteen cases tested positive for at least one identical type in their tumor and eyebrow hair samples (data not shown).

Associations between HPV DNA positivity in eyebrow hairs and case–control status stratified by HPV DNA status of the tumor are presented in Table 4. Among the 93 HPV DNA-positive cases, 75% (n = 70) tested DNA-positive for the same type in the eyebrow hairs as in the tumor (data not shown). For 18 of the HPV types tested, the DNA prevalence in the eyebrow hairs was significantly higher among cases with the same type detected in the tumor tissue when compared to controls; statistical significance was retained for 11 types after accounting for multiple comparisons. In contrast, no significant positive associations were observed between the presence of specific HPV types in eyebrow hairs and SCC cases who had tumors that were positive for HPV type(s) other than the type of interest detected in the eyebrow hair. Furthermore, no difference in DNA prevalence in eyebrow hairs was observed between controls and the 49 SCC cases with HPV DNAnegative tumors (Table 4). Thus, associations between HPV types in eyebrow hairs and SCC were specific to those cases with tumors positive for the identical HPV type(s).

Among the SCC cases who were concordant for a single HPV type in their eyebrow hair and tumor tissue, the mean viral DNA load (expressed as the number of viral copies per 100,000 cell equivalents) was greater in the eyebrow hairs than tumors for HPV5 (1,172 *vs*. 63), HPV8 (471 *vs*. 43), HPV24 (139 *vs*. 0.7) and HPV38 (160 *vs*. 54) (Table 5). Additionally, compared to controls, the mean viral DNA load in the eyebrow hairs among the cases was greater for HPV5 (1,172 *vs*. 256), HPV8 (471 *vs*. 75) and HPV24 (139 *vs*. 86) but lower for HPV38 (160 *vs*. 211) (Table 5). None of the samples tested by qPCR were positive for HPV20 and HPV36, and the eyebrow hair sample from a single SCC case was qPCR positive for HPV15 (532 copies/100,000 cell equivalents) (data not shown).

Results of the analysis modeled after Proby *et al*.[22] show that HPV seroreactivity (methods for HPV antibody measurement have been described previously[14]) is associated with SCC, regardless of the presence of HPV DNA in eyebrow hairs, although these associations were not statistically significant (Table 6, A). When the reference group was restricted to those who were negative for both HPV DNA and HPV antibodies, independent associations with SCC of similar magnitude were observed for the presence of HPV antibodies (OR = 1.92, 95% CI = 0.56–6.61) and HPV DNA (OR = 1.90, 95% CI = 0.62–5.77), although neither association was statistically significant (Table 6, B). Associations with SCC were of greater magnitude for the participants who were positive for both HPV DNA and antibodies,

whether they were positive for the same HPV types (OR = 2.37; 95% CI = 0.78–7.19) or discordant types (OR = 3.36, 95% CI = 1.12–10.07), with the latter association being statistically significant.

Discussion

Our findings are consistent with some but not all previous studies. Australian and Italian case–control studies[8,9] did not observe statistically significant associations between genus-beta HPV infection in eyebrow hairs and SCC. In contrast, case–control studies from The Netherlands reported associations consistent with our study findings.[6,7] In previous case–control studies measuring DNA in eyebrow hairs for the six EV-HPV types (5, 8, 15, 20, 24 and 38), type-specific associations among Dutch individuals were observed for HPV5, 15 and 20.[6,10] Differences in findings across studies could be due to differences in DNA detection methods and assay sensitivities, as well as underlying population characteristics that could affect cutaneous HPV infection and its association with SCC, such as ambient UVR exposure.

This is the first case–control study to investigate the associations between SCC and genus-beta HPV DNA in plucked eyebrow hairs incorporating comparisons with the presence of DNA in the tumor tissues. After correction for multiple comparisons, associations between HPV DNA in eyebrow hairs and SCC were statistically significant for eleven of 25 genusbeta types when comparing SCC cases with DNA for the same HPV type present in the tumor tissue to controls. Furthermore, no differences in HPV DNA prevalence in eyebrow hairs were observed when comparing SCC cases with tumors negative for all beta-HPV types to controls. Therefore, results from previous studies of HPV DNA in eyebrow hairs and SCC that did not take into account the presence of HPV DNA in the tumor tissues may have been attenuated. Given the correction for multiple comparisons and HPV type-specificity observed, it is not likely that the findings from this study are due to chance.

Positive associations with SCC were observed for HPV23 and HPV38 in analyses including eyebrow hairs only as well as analyses stratified by HPV DNA in the tumor. However, compared to cases, qPCR analysis revealed higher viral DNA loads in eyebrow hairs of controls for HPV38. For types 5, 8, and 24 case–control differences in DNA prevalence in eyebrow hairs were only observed, when accounting for HPV DNA in the tumor, but higher viral DNA loads were observed in cases compared to controls for these types. Some samples were positive by multiplex PCR but negative by qPCR, reflecting the lower analytic sensitivity of qPCR that resulted from lower amounts of input DNA used (2 μl *vs*. 10 μl), due the limited sample volumes available. Beta-globin levels did not differ between the SCC cases and controls. However, a greater proportion of controls were HPV qPCR negative compared to cases, further indicating that viral loads were lower in eyebrow hairs from controls compared to cases.

Within SCC cases, the mean viral DNA load was greater in eyebrow hairs than tumors for HPV5, 8, 24 and 38. One explanation may be that eyebrow hair follicle cells are more homogenous compared to tumor tissues which contain a mixture of cells (*i.e*., malignant and normal keratinocytes, infiltrating lymphocytes). Still, the absolute viral loads in tumor

tissues were orders of magnitude less than one copy per cell, suggesting that these tumors did not arise from clonal expansion of an HPV-infected cell. Previous studies have shown that cutaneous HPV loads are greater in actinic keratoses compared to SCC, suggesting that HPV may play a role in earlier stages in skin carcinogenesis.[21] Additionally, viral load may decrease during skin carcinogenesis due to disruption of cell differentiation, a condition necessary for completion of the viral lifecycle.[23]

Although the type-specific associations vary across study populations, similar type-specific associations are observed within this study across multiple biomarker measures of HPV infection. Previous reports from the same case–control study demonstrated an association between any genus-beta HPV seropositivity and SCC, with type-specific associations observed for HPV8 and HPV17.[14] Also, greater seroprevalence for HPV5, HPV17 and HPV24 was also observed for SCC cases with DNA in their tumor tissues for the same types. Similar findings were not observed when comparing genus-beta HPV DNA prevalence in eyebrow hairs between SCC cases and controls. However, when accounting for the presence of HPV DNA in the tumor, SCC cases with HPV5, HPV8, HPV17 and HPV24 DNA positive tumors had a higher DNA prevalence for the same type in their eyebrows compared to controls. Furthermore, viral DNA loads in eyebrow hairs were higher in cases for HPV5, HPV8 and HPV24 compared to controls.

An ongoing challenge in epidemiological research investigating the associations between cutaneous HPV infection and its potential role in SCC development is defining the presence of an HPV infection that is clinically relevant, especially as cutaneous HPV infection is ubiquitous in the general population. One method to address this issue is to compare HPV seroprevalences between SCC cases and controls in the absence or presence of concordant HPV DNA in the eyebrow hairs. Despite being limited by a small sample size, analyses in this study suggested that the independent associations with SCC were similar for HPV DNA in eyebrow hairs and HPV seropositivity. Furthermore, the magnitude of the association increased among participants who were positive for both DNA in the eyebrow hair and HPV antibodies. It is possible that the combination of biomarkers measures a higher risk cutaneous HPV infection with greater specificity than either of the biomarkers alone.[24] Although the risk estimate was greatest for the subgroup of participants who were seropositive for different HPV types than those detected in their eyebrow hairs, it was not significantly different from that observed in the group with concordant HPV types. Furthermore, the importance of type concordance when comparing antibodies and the presence of DNA is unclear, given that antibodies are a measure of past exposure. Prospective studies are needed to distinguish the differences in SCC risk associated with HPV infection as measured by different biomarkers.

As described previously,[14] the clinics used for recruitment service the same underlying community, with the controls demonstrating similar health behaviors as the general population. Therefore, it is believed that this study results are generalizable to external populations. The significant case–control differences in sex and age is another limitation of this study. However, results of the sensitivity analyses demonstrated that the observed associations between cutaneous HPV and SCC were not likely due to residual confounding by age and sex. Correction for multiple comparisons reduced the statistical significance of

the associations between SCC and DNA in the eyebrow hairs for HPV23 and HPV38, and as such, chance cannot be ruled out as an explanation for these findings. Even so, a majority of the associations stratified by HPV DNA status in the tumor remained significant after consideration for multiple comparisons.

This is the first study in a US population to investigate the association between genus-beta HPV DNA in plucked eyebrow hairs and SCC and to stratify these associations by the presence or absence of genus-beta HPV DNA in the tumor tissues. In conclusion, this case–control study provides continued evidence for the potential role of cutaneous HPV infections in SCC, evidenced by observations that cases, compared to controls, had a significantly higher prevalence of HPV DNA in their eyebrow hairs, with particularly strong type-specific associations observed among tumor DNApositive cases. In addition, qPCR analysis revealed differences in viral DNA loads by sample type and case–control status. Finally, similar type-specific associations were observed across biomarkers, including serological associations previously reported.[14] Natural history studies of cutaneous HPV infections are warranted to not only better understand the direction of the association between HPV infections and SCC, but to elucidate the potential for type-specific HPV infections in SCC development, or more simply, implicate any cutaneous HPV type infection as a risk factor for SCC.

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Abbreviations

CI	confidence interval

EBH	eyebrow hairs
EV	epidermodysplasia verruciformis
HPV	human papillomavirus
LCS	Moffitt's Lifetime Cancer Screening clinic
OR	odds ratio
qPCR	quantitative, real-time PCR
SCC	squamous cell carcinoma
TS-MPG	type-specific multiplex genotyping
USF	University of South Florida

What's new?

Some HPVs contribute to cervical cancer, but other types, called genus beta HPVs, commonly infect the skin and are associated with squamous cell carcinoma. In this study, the authors investigated the association between genus-beta HPV DNA found in plucked eyebrow hairs and the presence of SCC, as well as the presence of genus-beta HPV DNA in the tumor tissue. They found that the viruses were found in eyebrow hairs more often in those with cancer than those without, suggesting that the virus plays a role in cancer development, and could make a good therapeutic target.

Table 1

Demographic, lifestyle and skin cancer risk factors in SCC cases and controls

	Controls (*n* = 290)		SCC (*n* = 168)		
Variable	***n***	**(%)**	***n***	**(%)**	***p*-Value[1]**
Age in years (mean, [SD])	55.3	(11.7)	64.2	(10.0)	<0.0001[2]
Age					
18–39	27	(9.3)	4	(2.4)	<0.0001
40–49	52	(17.9)	10	(6.0)	
50–59	102	(35.2)	35	(20.8)	
60–69	81	(27.9)	64	(38.1)	
70–80	28	(9.7)	55	(32.7)	
Sex					
Female	181	(62.4)	57	(33.9)	<0.0001
Male	109	(37.6)	111	(66.1)	
Education					
>12 years	258	(89.9)	117	(78.0)	0.001
≤12 years	29	(10.1)	33	(22.0)	
Eye color					
Dark brown	79	(27.5)	19	(12.8)	0.003
Blue	81	(28.2)	61	(40.9)	
Green	48	(16.7)	23	(15.4)	
Hazel	45	(15.7)	30	(20.1)	

	Controls (*n* = 290)		SCC (*n* = 168)		
Variable	***n***	**(%)**	***n***	**(%)**	***p*-Value[1]**
Light brown	34	(11.9)	16	(10.7)	
Hair color					
Black/brown	227	(78.6)	98	(65.3)	0.003
Blonde/red	62	(21.5)	52	(34.7)	
Job in sun for ≥3 months					
No	210	(72.9)	75	(50.0)	<0.0001
Yes	78	(27.1)	75	(50.0)	
History of blistering sunburn					
No	91	(31.7)	35	(23.5)	0.07
Yes	196	(68.3)	114	(76.5)	
Cutaneous sensitivity to season's first sun exposure					
Tan or no change in skin color	42	(14.6)	20	(13.4)	0.001
Mild sunburn turns to a tan	132	(45.8)	44	(29.5)	
Sunburn with or without blisters	114	(39.6)	85	(57.1)	
Tanning ability to repeated sun exposure					
It tans easily	167	(58.6)	55	(36.9)	<0.0001
It can tan if you work at it	96	(33.7)	70	(47.0)	
It is unable to tan	22	(7.7)	24	(16.1)	
Alcohol consumption					
No drinks in past year	37	(12.9)	29	(19.5)	0.07

Variable	Controls (*n* = 290)		SCC (*n* = 168)		
	n	**(%)**	***n***	**(%)**	***p*-Value[1]**
≥1 drink in the past year	251	(87.2)	120	(80.5)	
Ever smoked 100 cigarettes					
No	144	(49.7)	46	(30.9)	0.0002
Yes	146	(50.3)	103	(69.1)	

This table presents and compares the distribution of demographic, lifestyle, and established skin cancer risk factors between SCC cases and controls.

Abbreviation: SD, standard deviation.

[1] *p*-Value for chi-square test.

[2] *p*-Value for Wilcoxon rank sum test.

Table 2

Associations between genus-beta type-specific HPV DNA prevalence in plucked eyebrow hairs and SCC

	Controls (*n* = 290)		All SCC cases (*n* = 168)							Age-restricted SCC cases *versus* controls (*n* = 109 SCC cases and 235 controls)[1]				
Genus-beta species/type	***n***	**(%)**	***n***	**(%)**	**OR[2]**	**(95% CI)[2]**	**OR[3]**	**(95% CI)[3]**	**p[4]**	**OR[2]**	**(95% CI)[2]**	**OR[3]**	**(95% CI)[3]**	**P[4]**
Any beta type														
Negative	77	(26.6)	22	(13.1)	1.00	(reference)	1.00	(reference)	ref.	1.00	(reference)	1.00	(reference)	ref.
Positive	213	(73.4)	146	(86.9)	1.75	(1.00–3.05)	1.68	(0.88–3.19)	0.12	1.94	(1.03–3.66)	1.65	(0.80–3.42)	0.18
Beta 1														
any	140	(48.3)	114	(67.9)	1.68	(1.09–2.59)	1.51	(0.92–2.48)	0.11	1.76	(1.07–2.89)	1.69	(0.95–3.01)	0.07
5	50	(17.2)	41	(24.4)	1.09	(0.65–1.81)	1.19	(0.66–2.16)	0.56	1.14	(0.62–2.06)	1.17	(0.57–2.40)	0.66
8	35	(12.1)	29	(17.3)	1.33	(0.74–2.38)	1.09	(0.55–2.18)	0.81	1.40	(0.72–2.69)	1.28	(0.58–2.83)	0.54
12	55	(19.0)	32	(19.0)	0.81	(0.47–1.40)	0.80	(0.42–1.52)	0.50	1.03	(0.55–1.93)	1.01	(0.48–2.16)	0.97
14	9	(3.1)	8	(4.8)	1.12	(0.39–3.25)	0.99	(0.28–3.41)	0.98	1.28	(0.36–4.56)	1.30	(0.29–5.81)	0.73
19	11	(3.8)	14	(8.3)	1.46	(0.60–3.55)	1.70	(0.62–4.65)	0.30	0.79	(0.26–2.46)	0.81	(0.22–3.00)	0.75
20	18	(6.2)	14	(8.3)	0.90	(0.40–2.03)	1.33	(0.53–3.33)	0.54	1.19	(0.45–3.12)	1.91	(0.63–5.84)	0.26
21	31	(10.7)	34	(20.2)	1.82	(1.01–3.28)	1.85	(0.92–3.70)	0.08	1.88	(0.94–3.76)	2.24	(0.99–5.10)	0.05
24	37	(12.8)	35	(20.8)	1.19	(0.68–2.10)	1.11	(0.58–2.12)	0.75	1.32	(0.68–2.58)	1.24	(0.57–2.66)	0.59
25	3	(1.0)	10	(6.0)	4.16	(1.02–16.90)	4.53	(1.00–20.70)	0.05	2.68	(0.56–12.90)	2.89	(0.52–16.10)	0.23
36	22	(7.6)	11	(6.5)	0.51	(0.23–1.15)	0.66	(0.26–1.68)	0.38	0.56	(0.20–1.55)	0.85	(0.26–2.79)	0.79
47	18	(6.2)	11	(6.5)	0.89	(0.38–2.05)	0.74	(0.27–2.01)	0.56	1.35	(0.55–3.32)	1.12	(0.38–3.33)	0.84
93	20	(6.9)	11	(6.5)	0.67	(0.29–1.53)	0.41	(0.15–1.14)	0.09	0.49	(0.17–1.44)	0.29	(0.07–1.14)	0.08
Beta 2														

	Controls (n = 290)		All SCC cases (n = 168)							Age-restricted SCC cases *versus* controls (n = 109 SCC cases and 235 controls)[1]				
Genus-beta species/type	***n***	**(%)**	***n***	**(%)**	**OR[2]**	**(95% CI)[2]**	**OR[3]**	**(95% CI)[3]**	**p[4]**	**OR[2]**	**(95% CI)[2]**	**OR[3]**	**(95% CI)[3]**	**P[4]**
any	164	(56.6)	119	(70.8)	1.50	(0.96–2.33)	1.67	(0.99–2.81)	0.05	1.76	(1.05–2.95)	1.75	(0.95–3.20)	0.07
9	30	(10.3)	22	(13.1)	0.99	(0.52–1.91)	1.05	(0.51–2.20)	0.89	1.24	(0.59–2.63)	1.28	(0.54–3.02)	0.57
15	17	(5.9)	10	(6.0)	1.05	(0.44–2.53)	0.94	(0.35–2.55)	0.90	1.11	(0.42–2.91)	1.00	(0.32–3.09)	0.99
17	42	(14.5)	37	(22.0)	1.22	(0.71–2.09)	1.23	(0.67–2.27)	0.50	1.15	(0.61–2.15)	1.08	(0.51–2.26)	0.85
22	21	(7.2)	17	(10.1)	0.88	(0.42–1.87)	0.97	(0.40–2.37)	0.95	1.42	(0.55–3.68)	2.22	(0.73–6.77)	0.16
23	65	(22.4)	52	(31.0)	1.36	(0.84–2.18)	1.90	(1.10–3.30)	0.02	1.72	(1.01–2.93)	1.94	(1.03–3.63)	0.04
37	39	(13.4)	28	(16.7)	1.22	(0.68–2.19)	1.28	(0.65–2.49)	0.48	1.20	(0.62–2.31)	1.15	(0.53–2.51)	0.72
38	52	(17.9)	55	(32.7)	1.76	(1.08–2.87)	1.84	(1.04–3.24)	0.04	2.14	(1.23–3.71)	2.10	(1.09–4.06)	0.03
80	39	(13.4)	28	(16.7)	1.11	(0.62–1.99)	1.17	(0.60–2.31)	0.64	1.27	(0.65–2.48)	1.07	(0.47–2.44)	0.88
Beta 3														
any	52	(17.9)	39	(23.2)	1.03	(0.62–1.73)	1.16	(0.64–2.10)	0.62	1.44	(0.81–2.58)	1.50	(0.75–2.99)	0.25
49	7	(2.4)	6	(3.6)	1.18	(0.35–3.98)	1.03	(0.25–4.18)	0.97	1.29	(0.30–5.51)	0.72	(0.10–5.25)	0.74
75	13	(4.5)	10	(6.0)	1.43	(0.55–3.71)	1.35	(0.42–4.29)	0.61	1.75	(0.64–4.84)	1.42	(0.37–5.46)	0.61
76	37	(12.8)	27	(16.1)	0.96	(0.53–1.73)	1.05	(0.53–2.07)	0.88	1.33	(0.68–2.60)	1.45	(0.66–3.21)	0.35
Beta 4														
92	6	(2.1)	10	(6.0)	1.81	(0.61–5.36)	2.39	(0.66–8.68)	0.19	2.68	(0.73–9.84)	4.12	(0.83–20.40)	0.08
Beta 5														
96	10	(3.4)	9	(5.4)	1.44	(0.50–4.16)	2.62	(0.78–8.83)	0.12	2.40	(0.73–7.89)	4.99	(1.27–19.70)	0.02
# of beta types														

	Controls (*n* = 290)		All SCC cases (*n* = 168)							Age-restricted SCC cases *versus* controls (*n* = 109 SCC cases and 235 controls)[1]				
Genus-beta species/type	***n***	**(%)**	***n***	**(%)**	**OR[2]**	**(95% CI)[2]**	**OR[3]**	**(95% CI)[3]**	**p[4]**	**OR[2]**	**(95% CI)[2]**	**OR[3]**	**(95% CI)[3]**	**P[4]**
1 type	64	(22.1)	32	(19.1)	1.61	(0.81–3.20)	1.41	(0.64–3.08)	0.40	1.65	(0.75–3.65)	1.22	(0.49–3.02)	0.67
2 types	56	(19.3)	24	(14.3)	1.12	(0.55–2.30)	1.30	(0.57–3.04)	0.55	1.12	(0.48–2.58)	1.14	(0.43–3.05)	0.79
3 types	26	(9.0)	26	(15.5)	2.70	(1.23–5.90)	1.62	(0.64–4.10)	0.31	2.96	(1.18–7.44)	1.60	(0.54–4.77)	0.40
≥4 types	67	(23.1)	64	(38.1)	2.02	(1.07–3.80)	2.22	(1.07–4.61)	0.03	2.53	(1.23–5.18)	2.51	(1.09–5.77)	0.03
					p_{trend} = 0.02		p_{trend} = 0.03			p_{trend} = 0.006		p_{trend} = 0.02		

This table presents the odds ratios and 95% confidence intervals for the independent associations between genus-beta type-specific HPV DNA prevalence in plucked eyebrow hairs and SCC. This table also presents *p*-values reflecting the significance of multiple comparisons based on the Bonferroni method of correction.

[1] Analyses restricted to participants 40–69 years of age. The age restricted analysis included 109 SCC cases and 235 controls.

[2] Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age and sex.

[3] OR and 95% CI adjusted for age, sex, education, hair color, occupational sunlight exposure, tanning ability and ever smoking at least 100 cigarettes in lifetime.

[4] *p*-Value for beta coefficient corresponding to the HPV term in the logistic regression model; statistical significance threshold: $p<0.002$.

Abbreviation: ref., reference.

Table 3

Genus-beta type-specific HPV DNA prevalence in SCC tumor tissues (*n* = 180)

	All (*n* = 180)	
Genus-beta species/type	***n***	**(%)**
Any beta type	118	(65.6)
Beta 1		
any	79	(43.9)
5	27	(15.0)
8	19	(10.6)
12	18	(10.0)
14	4	(2.2)
19	10	(5.6)
20	13	(7.2)
21	9	(5.0)
24	19	(10.6)
25	0	(0.0)
36	20	(11.1)
47	1	(0.6)
93	8	(4.4)
Beta 2		
any	100	(55.6)
9	9	(5.0)
15	20	(11.1)
17	23	(12.8)
22	19	(10.6)
23	49	(27.2)
37	6	(20.6)
38	31	(17.2)
80	22	(12.2)
Beta 3		
75	6	(3.3)
76	0	(0.0)
Beta 4		
92	10	(5.6)
Beta 5		
96	8	(4.4)
# of beta types		
1 type	38	(21.1)
2 types	23	(12.8)
3 types	23	(12.8)

	All (*n* = 180)	
Genus-beta species/type	***n***	**(%)**
≥4 types	34	(18.9)

Table 4

Association between genus-beta type-specific HPV DNA prevalence in plucked eyebrow hairs and SCC, stratified by DNA status of the SCC tumor tissue for the same HPV type

			SCC cases by HPV DNA status of tumor											
			Positive for at least one HPV type (*n* = 93)								Negative for			
	Controls (*n* = 290)		HPV-positive for specific type[2]					HPV-negative for specific type[3]			all HPV types (*n* = 49)			
Eyebrow hair HPV DNA results by type[1]	*n*	(%)	*n*	(%)	OR[4]	(95% CI)[4]	*p*[5]	*n*	(%)	OR[4]	(95% CI)[4]	*n*	(%)	OR[4]	(95% CI)[4]
Any beta type															
Negative	77	(26.6)	10	(10.7)	1.00	(reference)	0.05	Not applicable since all types are combined in the HPV-positive group				10	(20.4)	1.00	(reference)
Positive	213	(73.4)	83	(89.3)	2.13	(0.99–4.50)						39	(79.6)	1.12	(0.52–2.40)
HPV 5															
Negative	240	(82.8)	6	(33.3)	1.00	(reference)	0.0003	62	(82.7)	1.00	(reference)	40	(81.6)	1.00	(reference)
Positive	50	(17.2)	12	(66.7)	7.25	(2.51–20.90)		13	(17.3)	0.75	(0.36–1.56)	9	(18.4)	0.78	(0.35–1.78)
HPV 8															
Negative	255	(87.9)	8	(50.0)	1.00	(reference)	0.003	65	(84.4)	1.00	(reference)	45	(91.8)	1.00	(reference)
Positive	35	(12.1)	8	(50.0)	5.52	(1.81–16.80)		12	(15.6)	1.12	(0.51–2.44)	4	(8.2)	0.62	(0.20–1.87)
HPV 12															
Negative	235	(81.0)	3	(21.4)	1.00	(reference)	0.0004	65	(82.3)	1.00	(reference)	44	(89.8)	1.00	(reference)
Positive	55	(19.0)	11	(78.6)	12.20	(3.08–47.9)		14	(17.7)	0.81	(0.40–1.65)	5	(10.2)	0.41	(0.15–1.11)
HPV 14															
Negative	281	(96.9)	0	(0.0)	–	–	–	88	(97.8)	1.00	(reference)	46	(93.9)	1.00	(reference)
Positive	9	(3.1)	3	(100.0)	–	–		2	(2.2)	0.58	(0.11–3.04)	3	(6.1)	1.69	(0.42–6.70)

Eyebrow hair HPV DNA results by type[1]	Controls (*n* = 290)		SCC cases by HPV DNA status of tumor: Positive for at least one HPV type (*n* = 93)									Negative for all HPV types (*n* = 49)			
			HPV-positive for specific type[2]					HPV-negative for specific type[3]							
	n	(%)	*n*	(%)	OR[4]	(95% CI)[4]	*p*[5]	*n*	(%)	OR[4]	(95% CI)[4]	*n*	(%)	OR[4]	(95% CI)[4]
HPV 19															
Negative	279	(96.2)	5	(55.6)	1.00	(reference)	0.0001	79	(94.0)	1.00	(reference)	47	(95.9)	1.00	(reference)
Positive	11	(3.8)	4	(44.4)	20.90	(4.47–97.30)		5	(6.0)	1.10	(0.33–3.73)	2	(4.1)	0.84	(0.17–4.08)
HPV 20															
Negative	272	(93.8)	9	(81.8)	1.00	(reference)	0.49	73	(89.0)	1.00	(reference)	48	(98.0)	1.00	(reference)
Positive	18	(6.2)	2	(18.2)	1.85	(0.33–10.50)		9	(11.0)	1.06	(0.41–2.76)	1	(2.0)	0.21	(0.03–1.70)
HPV 21															
Negative	259	(89.3)	2	(28.6)	1.00	(reference)	0.0002	68	(79.1)	1.00	(reference)	44	(89.8)	1.00	(reference)
Positive	31	(10.7)	5	(71.4)	28.35	(4.77–168.47)		18	(20.9)	1.85	(0.89–3.84)	5	(10.2)	0.41	(0.15–1.11)
HPV 24															
Negative	253	(87.2)	2	(11.8)	1.00	(reference)	<0.0001	66	(86.8)	1.00	(reference)	44	(89.8)	1.00	(reference)
Positive	37	(12.8)	15	(88.2)	35.60	(7.45–170.00)		10	(13.2)	0.55	(0.23–1.25)	5	(10.2)	0.58	(0.21–1.61)
HPV 36															
Negative	268	(92.4)	11	(61.1)	1.00	(reference)	0.01	74	(98.7)	1.00	(reference)	47	(95.9)	1.00	(reference)
Positive	22	(7.6)	7	(38.9)	4.66	(1.54–14.10)		1	(1.3)	0.09	(0.01–0.70)	2	(4.1)	0.33	(0.07–1.51)
HPV 47															
Negative	272	(93.8)	0	(0.0)	–	–	–	87	(94.6)	1.00	(reference)	47	(95.9)	1.00	(reference)
Positive	18	(6.2)	1	(100.0)	–	–		5	(5.4)	0.54	(0.18–1.64)	2	(4.1)	0.53	(0.11–2.42)

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Eyebrow hair HPV DNA results by type[1]	Controls (*n* = 290)		SCC cases by HPV DNA status of tumor: Positive for at least one HPV type (*n* = 93)									Negative for all HPV types (*n* = 49)			
			HPV-positive for specific type[2]					HPV-negative for specific type[3]							
	n	(%)	*n*	(%)	OR[4]	(95% CI)[4]	*p*[5]	*n*	(%)	OR[4]	(95% CI)[4]	*n*	(%)	OR[4]	(95% CI)[4]
HPV 93															
Negative	270	(93.1)	4	(57.1)	1.00	(reference)	0.04	81	(94.2)	1.00	(reference)	47	(95.9)	1.00	(reference)
Positive	20	(6.9)	3	(42.9)	5.56	(1.07–29.10)		5	(5.8)	0.52	(0.18–1.55)	2	(4.1)	0.49	(0.11–2.23)
HPV 9															
Negative	260	(89.7)	3	(42.9)	1.00	(reference)	0.01	76	(88.4)	1.00	(reference)	46	(93.9)	1.00	(reference)
Positive	30	(10.3)	4	(57.1)	9.42	(1.72–51.60)		10	(11.6)	0.79	(0.34–1.86)	3	(6.1)	0.46	(0.13–1.63)
HPV 15															
Negative	273	(94.1)	9	(69.2)	1.00	(reference)	0.002	76	(95.0)	1.00	(reference)	48	(98.0)	1.00	(reference)
Positive	17	(5.9)	4	(30.8)	9.29	(2.23–38.70)		4	(5.0)	1.22	(0.37–4.08)	1	(2.0)	0.39	(0.05–3.06)
HPV 17															
Negative	248	(85.5)	11	(55.0)	1.00	(reference)	0.04	62	(84.9)	1.00	(reference)	40	(81.6)	1.00	(reference)
Positive	42	(14.5)	9	(45.0)	2.94	(1.05–8.21)		11	(15.1)	0.76	(0.35–1.68)	9	(18.4)	1.02	(0.44–2.33)
HPV 22															
Negative	269	(92.8)	5	(33.3)	1.00	(reference)	<0.0001	75	(96.2)	1.00	(reference)	48	(98.0)	1.00	(reference)
Positive	21	(7.2)	10	(66.7)	21.70	(6.38–74.00)		3	(3.8)	0.21	(0.05–0.81)	1	(2.0)	0.17	(0.02–1.35)
HPV 23															
Negative	225	(77.6)	18	(48.6)	1.00	(reference)	0.001	44	(78.6)	1.00	(reference)	35	(71.4)	1.00	(reference)
Positive	65	(22.4)	19	(51.4)	3.44	(1.62–7.30)		12	(21.4)	0.74	(0.34–1.62)	14	(28.6)	1.28	(0.63–2.58)

			SCC cases by HPV DNA status of tumor											
			Positive for at least one HPV type (*n* = 93)								Negative for			
	Controls (*n* = 290)		HPV-positive for specific type[2]					HPV-negative for specific type[3]			all HPV types (*n* = 49)			
Eyebrow hair HPV DNA results by type[1]	*n*	(%)	*n*	(%)	OR[4]	(95% CI)[4]	*p*[5]	*n*	(%)	OR[4]	(95% CI)[4]	*n*	(%)	OR[4]	(95% CI)[4]
HPV 37															
Negative	251	(86.6)	0	(0.0)	–	–	–	71	(78.9)	1.00	(reference)	46	(93.9)	1.00	(reference)
Positive	39	(13.4)	3	(100.0)	–	–		19	(21.1)	1.72	(0.85–3.48)	3	(6.1)	0.45	(0.13–1.53)
HPV 38															
Negative	238	(82.1)	8	(33.3)	1.00	(reference)	0.0002	51	(73.9)	1.00	(reference)	35	(71.4)	1.00	(reference)
Positive	52	(17.9)	16	(66.7)	6.31	(2.39–16.60)		18	(26.1)	1.08	(0.53–2.19)	14	(28.6)	1.50	(0.73–3.08)
HPV 80															
Negative	251	(86.6)	6	(35.3)	1.00	(reference)	<0.0001	67	(88.2)	1.00	(reference)	44	(89.8)	1.00	(reference)
Positive	39	(13.4)	11	(64.7)	9.90	(3.20–30.60)		9	(11.8)	0.80	(0.34–1.84)	5	(10.2)	0.65	(0.23–1.79)
HPV 75															
Negative	277	(95.5)	1	(25.0)	1.00	(reference)	0.001	85	(95.5)	1.00	(reference)	47	(95.9)	1.00	(reference)
Positive	13	(4.5)	3	(75.0)	76.50	(6.67–878.00)		4	(4.5)	0.87	(0.23–3.29)	2	(4.1)	0.93	(0.19–4.52)
HPV 92															
Negative	284	(97.9)	3	(37.5)	1.00	(reference)	<0.0001	84	(98.8)	1.00	(reference)	47	(95.9)	1.00	(reference)
Positive	6	(2.1)	5	(62.5)	58.60	(9.58–358.00)		1	(1.2)	0.30	(0.03–2.84)	2	(4.1)	1.32	(0.25–7.09)
HPV 96															
Negative	280	(96.6)	4	(66.7)	1.00	(reference)	0.01	83	(95.4)	1.00	(reference)	46	(93.9)	1.00	(reference)
Positive	10	(3.4)	2	(33.3)	11.80	(1.81–77.30)		4	(4.6)	1.20	(0.29–4.94)	3	(6.1)	1.59	(0.39–6.54)

This table presents the odds ratios and 95% confidence intervals for the independent, case–control associations between genus-beta type-specific HPV DNA eyebrow specimens, stratified by the HPV DNA status of the same HPV type in the SCC tumor tissue.

[1] No tumor specimens were DNA positive for HPV types 25, 49, and 76. Therefore, concordance analyses were not conducted for these HPV types.

[2] Number of SCC cases with HPV DNA-positive tumors varies by the type of interest indicated in the first column.

[3] Number of SCC cases with tumors negative for the type of interest indicated in the first column (*i.e.*, these cases may be positive for other HPV types).

[4] Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age and sex.

[5] p-Value for beta coefficient corresponding to the HPV term in the logistic regression model; statistical significance threshold: $p < 0.002$.

Table 5

Genus-beta type-specific DNA loads in SCC tumor tissues and in paired plucked eyebrow hairs in SCC cases and controls

						Copy numbers among qPCR+		
Beta-globin/ HPV/sample type	**Participant status**	**Multiplex PCR + *n***	**qPCR – *n***	**Threshold of detection range**	**Paired samples qPCR + *n*[1]**	**Mean[2]**	**(S.D.)**	**Range**
Beta-globin								
Tumor	Case	31	0	–	30	165,609	(123,029)	1,590–520,000
Eyebrow	Case	31	1	–	30	4,753	(5,467)	182–25,900
Eyebrow	Control	56	0	–	56	3,707	(3,824)	124–23,800
HPV 5								
Tumor	Case	4	1	<1.3	3	63	(70)	15–143
Eyebrow	Case	4	1	<148	3	1,172	(884)	361–2,115
Eyebrow	Control	5	4	<26 to <133	1	256	–	–
HPV 8								
Tumor	Case	5	3	<1.0 to <1.1	2	43	(36)	17–69
Eyebrow	Case	5	2	<106 to <138	2	471	(396)	191–751
Eyebrow	Control	5	4	<8 to <63	1	75	–	–
HPV 23								
Tumor	Case	8	8	<0.4 to <8	0	–	–	–
Eyebrow	Case	8	7[3]	<33 to <174	0	–	–	–
Eyebrow	Control	20	17	<26 to <453	3	153	(71)	82–227
HPV 24								
Tumor	Case	8	6	<0.6 to <126	2	0.7	(0.1)	0.7–0.8
Eyebrow	Case	8	3	<12 to <60	2	139	(126)	50–229
Eyebrow	Control	8	5	<43 to <465	3	86	(52)	27–125
HPV 38								
Tumor	Case	5	3	<0.7 to <1.7	2	54	(73)	2.3–106
Eyebrow	Case	5	1	<23	2	160	(157)	49–270
Eyebrow	Control	12	6	<23 to <1,613	6	211	(144)	58–428

This table presents the mean viral DNA load (expressed as the number of viral copies per 100,000 cell equivalents) in tumor tissue and eyebrow hair specimens among the SCC cases who were concordant for a single HPV type in their eyebrow hair and tumor tissue. For comparison, this table also presents the mean viral DNA load in eyebrow hair specimens from controls with a single HPV infection in their eyebrows hairs for those types positive in the specimens of the SCC cases.

[1]Counts used to estimate mean and standard deviation (S.D.); included SCC cases with both eyebrow and tumor samples that were qPCR+.

[2]Viral DNA loads expressed as the number of viral copies per 100,000 cell equivalents.

[3]One eyebrow hair sample from a SCC case tested negative for beta-globin by qPCR.

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Table 6

Associations between genus-beta HPV seropositivity and SCC in the absence or presence of concordant genus-beta HPV DNA in plucked eyebrow hairs (A) and associations between the presence of genus-beta HPV DNA in plucked eyebrow hairs and SCC in the absence or presence of antibodies to the same or discordant HPV types (B).

Genus-beta HPV seropositivity and viral DNA status of eyebrow (EB) hairs	Controls (*n* = 290) *n* (%)	SCC (*n* = 168)		
		n (%)	OR	(95% CI)[1]
A[2]				
Seronegative regardless of EB DNA	117 (40.3)	46 (27.4)	1.00	(reference)
Seropositive without concordant EB DNA	110 (37.9)	64 (38.1)	1.73	(0.97–3.10)
Seropositive with concordant EB DNA	63 (21.7)	58 (34.5)	1.42	(0.75–2.68)
B				
Seronegative/EB DNA negative	36 (12.4)	5 (3.0)	1.00	(reference)
Seropositive/EB DNA negative	41 (14.1)	17 (10.1)	1.92	(0.56–6.61)
Seronegative/EB DNA positive	81 (27.9)	41 (24.4)	1.90	(0.62–5.77)
Seropositive/EB DNA positive for discordant types	69 (23.8)	47 (28.0)	3.36	(1.12–10.07)
Seropositive/ EB DNA positive for concordant types	63 (21.7)	58 (34.5)	2.37	(0.78–7.19)

[1] Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, sex, education, hair color, occupational sunlight exposure, tanning ability and ever smoking at least 100 cigarettes in lifetime.

[2] Analysis modeled after Proby *et al.* Am J Transplant 2011.